1: Pediatrics. 2004 May;113(5):e448-57.

Long-term intravenous treatment of Pompe disease with recombinant human alpha-glucosidase from milk.

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OBJECTIVE: Recent reports warn that the worldwide cell culture capacity is insufficient to fulfill the increasing demand for human protein drugs. Production in milk of transgenic animals is an attractive alternative. Kilogram quantities of product per year can be obtained at relatively low costs, even in small animals such as rabbits. We tested the long-term safety and efficacy of recombinant human -glucosidase (rhAGLU) from rabbit milk for the treatment of the lysosomal storage disorder Pompe disease. The disease occurs with an estimated frequency of 1 in 40,000 and is designated as orphan disease. The classic infantile form leads to death at a median age of 6 to 8 months and is diagnosed by absence of alpha-glucosidase activity and presence of fully deleterious mutations in the alpha-glucosidase gene. Cardiac hypertrophy is characteristically present. Loss of muscle strength prevents infants from achieving developmental milestones such as sitting, standing, and walking. Milder forms of the disease are associated with less severe mutations and partial deficiency of alpha-glucosidase. METHODS: In the beginning of 1999, 4 critically ill patients with infantile Pompe disease (2.5-8 months of age) were enrolled in a single-center open-label study and treated intravenously with rhAGLU in a dose of 15 to 40 mg/kg/week. RESULTS: Genotypes of patients were consistent with the most severe form of Pompe disease. Additional molecular analysis failed to detect processed forms of alpha-glucosidase (95, 76, and 70 kDa) in 3 of the 4 patients and revealed only a trace amount of the 95-kDa biosynthetic intermediate form in the fourth (patient 1). With the more sensitive detection method, 35S-methionine incorporation, we could detect low-level synthesis of -glucosidase in 3 of the 4 patients (patients 1, 2, and 4) with some posttranslation

modification from 110 kDa to 95 kDa in 1 of them (patient 1). One patient (patient 3) remained totally deficient with both detection methods (negative for cross-reactive immunologic material [CRIM negative]). The alpha-glucosidase activity in skeletal muscle and fibroblasts of all 4 patients was below the lower limit of detection (<2% of normal). The rhAGLU was tolerated well by the patients during >3 years of treatment. Anti-rhAGLU immunoglobulin G titers initially increased during the first 20 to 48 weeks of therapy but declined thereafter. There was no consistent difference in antibody formation comparing CRIM-negative with CRIM-positive patients. Muscle alpha-glucosidase activity increased from <2% to 10% to 20% of normal in all patients during the first 12 weeks of treatment with 15 to 20 mg/kg/week. For optimizing the effect, the dose was increased to 40 mg/kg/week. This resulted, 12 weeks later, in normal alpha-glucosidase activity levels, which were maintained until the last measurement in week 72. Importantly, all 4 patients, including the patient without any endogenous alpha-glucosidase (CRIM negative), revealed mature 76- and 70-kDa forms of -glucosidase on Western blot. Conversion of the 110-kDa precursor from milk to mature 76/70-kDa alpha-glucosidase provides evidence that the enzyme is targeted to lysosomes, where this proteolytic processing occurs. At baseline, patients had severe glycogen storage in the quadriceps muscle as revealed by strong periodic acid-Schiff--positive staining and lacework patterns in hematoxylin and eosin--stained tissue sections. The muscle pathology correlated at each time point with severity of signs. Periodic acid-Schiff intensity diminished and number of vacuoles increased during the first 12 weeks of treatment. Twelve weeks after dose elevation, we observed signs of muscle regeneration in 3 of the 4 patients. Obvious improvement of muscular architecture was seen only in the patient who learned to walk. Clinical effects were significant. All patients survived beyond the age of 4 years, whereas untreated patients succumb at a median age of 6 to 8 months. The characteristic cardiac hypertrophy present at start of treatment diminished significantly. The left ventricular mass index decreased from 171 to 599 g/m2 (upper limit of normal 86.6 g/m2 for infants from 0 to 1 year) to 70 to 160 g/m2 during 84 weeks of treatment. In addition, we found a significant change of slope for the diastolic thickness of the left ventricular posterior wall against time at t = 0 for each separate patient. Remarkably, the younger patients (patients 1 and 3) showed no significant respiratory problems during the first 2 years of life. One of the younger patients recovered from a life-threatening bronchiolitis at the age of 1 year without sequelae, despite borderline oxygen saturations at inclusion. At the age of 2, however, she became ventilator dependent after surgical removal of an infected Port-A-Cath. She died at the age of 4 years and 3 months suddenly after a short period of intractable fever of >42 degrees C, unstable blood pressure, and coma. The respiratory course of patient 1 remained uneventful. The 2 older patients, who both were hypercapnic (partial pressure of carbon dioxide: 10.6 and 9.8 kPa; normal range: 4.5-6.8 kPa) at start of treatment, became ventilator dependent before the first infusion (patient 2) and after 10 weeks of therapy (patient 4). Patient 4 was gradually weaned from the ventilator after 1 year of high-dose treatment and was eventually completely ventilator-free for 5 days, but this situation could not be maintained. Currently, both patients are completely ventilator dependent. The most remarkable progress in motor function was seen in the younger patients (patients 1 and 3). They achieved motor milestones that are unmet in infantile Pompe disease. Patient 1 learned to crawl (12 months), walk (16 months), squat (18 months), and climb stairs (22 months), and patient 3 learned to sit unsupported. The Alberta Infant Motor Scale score for patients 2, 3, and 4 remained far below p5. Patient 1 followed the p5 of normal. CONCLUSION:

Our study shows that a safe and effective medicine can be produced in the milk of mammals and encourages additional development of enzyme replacement therapy for the several forms of Pompe disease. Restoration of skeletal muscle function and prevention of pulmonary insufficiency require dosing in the range of 20 to 40 mg/kg/week. The effect depends on residual muscle function at the start of treatment. Early start of treatment is required.

PMID: 15121988 [PubMed - indexed for MEDLINE]
